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Transient inositol 1,4,5-trisphosphate-induced Ca^{2+} release: A model based on regulatory Ca^{2+}-binding sites along the permeation pathway

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ABSTRACT **A remarkable property of Ca^{2+} fluxes through the inositol 1,4,5-trisphosphate ($InsP_3$)-gated Ca^{2+} channel is that successive increments of $InsP_3$ induce repeated transient release of accumulated Ca^{2+}. The initial aim of this study was to propose a model, based on hypotheses compatible with the current description of this Ca^{2+} channel, which could account for such experimental observations. The key feature of the model was the assumption that the Ca^{2+}-binding sites on the receptor, whose occupancy leads to immediate channel activation but to subsequent slow channel desensitization, were located somewhere along the permeation pathway and were therefore sensitive to the flux of Ca^{2+} rather than the cytosolic or luminal Ca^{2+} concentration *per se*. Simulation showed that, provided Ca^{2+} bound to both activating and inhibitory sites with adequate cooperativity, addition of submaximal concentrations $InsP_3$ resulted in transient opening well above the stationary state. The model also rationalized the documented existence of a threshold for $InsP_3$ action, the puzzling control of channel sensitivity to $InsP_3$ by luminal and cytosolic Ca^{2+}, as well as the functional heterogeneity of the Ca^{2+} pools.**

Several studies of inositol 1,4,5-trisphosphate ($InsP_3$)-induced Ca^{2+} release have reported that a low $InsP_3$ concentration was unable to release the total Ca^{2+} content of $InsP_3$-sensitive pools (1–4). The fact that subsequent addition of a maximal dose of $InsP_3$ finally empties the Ca^{2+} pool excluded the possibility that this phenomenon was due to classical desensitization, leading to a closed channel form with high affinity for the agonist. To account for such partial release, heterogeneity of the Ca^{2+} pools with respect to $InsP_3$ sensitivity was first invoked, each of these pools being released in an all-or-none manner (4). This partial discharge of the population of pools was therefore described as "quantal release" (1). The concept of incremental detection was then introduced to describe the fact that successive minute increases in $InsP_3$ (even smaller than 2-fold increases) provoked sequential Ca^{2+} discharges (3). It was recently demonstrated (5) that incremental detection could indeed be explained, assuming a heterogeneous population of functionally distinct $InsP_3$-sensitive Ca^{2+} pools, each with its own receptor isoform characterized by highly positive cooperativity in $InsP_3$ response.

As the hypotheses underlying such analysis are rather demanding, alternative models explored whether transient $InsP_3$-induced opening of the channels in a homogeneous population of the Ca^{2+} pools could be the basis for partial pool discharge. An initially developed "steady state" model considered that the Ca^{2+} release stopped when the luminal Ca^{2+} fell to low concentrations (6), but experiments designed to test this model gave contradictory results (3, 7–10). A subsequent proposal, based on related observations with the ryanodine receptor, suggested that the channel "adapted" to submaximal stimuli (11), but this proposal has not yet been tested for the $InsP_3$ receptor. Independently, a theoretical analysis suggested that the mechanism of incremental detection, if present at the level of a single pool, required some sort of energy-consuming process (12), but the "memory" molecule postulated in this study was not identified. Thus, the initial challenge for us was to develop a model based on acceptable hypotheses that could explain partial Ca^{2+} release and incremental $InsP_3$ detection for a homogeneous population of receptors.

The main guiding ideas in the elaboration of this model were that the required energy was contributed by the Ca^{2+} gradient between the lumen and the cytosol. To account for transient repetitive activation of the channel, it was imagined that both activating and inhibiting Ca^{2+}-binding sites on the $InsP_3$ receptor, responsible for fast activation and slow desensitization (13–15), respectively, were situated downstream from the gate, inside what can be considered as a distinct intermediate domain, separate both from the luminal and the cytosolic compartments. Analysis of a simple version of this model revealed very appealing features, as it not only explained many observed facts but also explained how these might have remained undetected under different conditions.

RATIONALE AND SIMULATION

Qualitative Description of the Model. Our model first relies on a few simple, generally accepted hypotheses. The $InsP_3$ receptor Ca^{2+} channel is a homotetramer that may bind up to four $InsP_3$ molecules with no apparent cooperativity (4, 16). $InsP_3$-induced Ca^{2+} release presents a bell-shaped dependence on cytosolic Ca^{2+} (13–15), but the reversible inhibiting action of Ca^{2+} on the $InsP_3$-stimulated Ca^{2+} efflux develops more slowly than the Ca^{2+} activation response (14, 15). A first version of this model was developed on the basis of several assumptions (Fig. 1), some of them essential (shown in italic), the other ones introduced for the sake of simplicity.

(*i*) *Ca^{2+} may bind to the $InsP_3$ receptor (R) at distinct activating or inhibitory sites.* Binding to them is mutually exclusive. Three forms of receptor therefore exist, R, R_a, and R_i. $InsP_3$ may bind to all of them (IR), with an affinity that we assume the same for all three forms (IR, IR_a, IR_i).

(*ii*) The channel may open only if it is in the IR_a state. *With Ca^{2+} bound to the inhibitory site (R_i, IR_i), the channel is desensitized.*

(*iii*) Binding of $InsP_3$ and Ca^{2+} to the activating site are always at equilibrium, but *binding of Ca^{2+} to the inhibitory*

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Abbreviation: $InsP_3$, inositol 1,4,5-trisphosphate.

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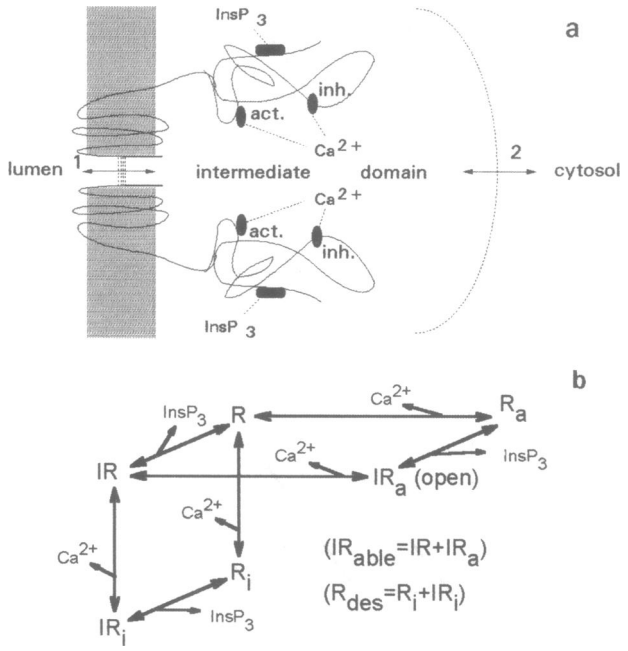

FIG. 1. Definition of the model. (*a*) Hypothetical spatial description of the channel. The permeation pathway delimits an intermediate compartment separated from the lumen by InsP_3 and Ca^{2+}-dependent gate 1 and from the bulk cytosol by the fictitious barrier 2. The activating (act.) and inhibitory (inh.) Ca^{2+}-binding sites sense the Ca^{2+} concentration in this intermediate domain. (*b*) Binding reaction scheme. Ca^{2+} in the intermediate domain binds to the activating sites (horizontal arrows) or to the inhibitory sites (vertical arrows) of both InsP_3-free receptor (R) and InsP_3-bound receptor (IR). IR_a is the open form of the channel.

site develops slowly (alternatively, we could have modeled fast binding but slow transition to the inhibitory state).

(*iv*) *The Ins*P_3*-controlled gate is situated on the transmembrane part of the channel. The* Ca^{2+}*-binding sites are located in an intermediate compartment defined downstream from the gate along the permeation pathway*. This compartment is considered as a macroscopic domain in which a homogeneous Ca^{2+} concentration is defined (this is obviously an oversimplified but useful description) and is probed by the binding sites.

Quantitative Description of the Model. The following notations will be used throughout the study. I is the free InsP_3 concentration. C_{lumen}, C_{dom}, and C_{cyto} refer to the free Ca^{2+} concentrations in the lumen, in the intermediate domain, and in the cytosol, respectively. R, IR, R_a, IR_a, R_i, and IR_i are the fractions of receptors being in these different respective states.

IR_{tot} is the fraction of InsP_3-bound receptors ($= IR + IR_a + IR_i$). At equilibrium, IR_{tot} is expressed as a Michaelian function of InsP_3 concentration characterized by the apparent equilibrium dissociation constant K. Binding of Ca^{2+} to the activating and inhibitory Ca^{2+}-binding sites is assumed to be cooperative, with apparent equilibrium dissociation constants K_{act} and K_{inh} and Hill coefficients n_a and n_i.

Because Ca^{2+} binding to the desensitizing site is supposed to be slower than the other binding reactions, a kinetic equation is introduced to describe how the fraction of desensitized receptor R_{des} ($= Ri + IR_i$) varies with respect to time:

$$\frac{dR_{des}}{dt} = k_+ C_{dom}^{n_i} (R + IR) - k_- R_{des}, \quad [1]$$

where k_+ and k_- are the kinetic constants of Ca^{2+} association to and dissociation from the inhibiting site, respectively. By definition, $K_{inh}^{n_i} = k_-/k_+$. In the stationary situation, the fraction of desensitized receptors is given by

$$R_{des}^{(stationary)} = \frac{1}{1 + \frac{K_{inh}^{n_i}}{C_{dom}^{n_i}}\left(1 + \frac{C_{dom}^{n_a}}{K_{act}^{n_a}}\right)}. \quad [2]$$

Although the fraction of active (open) receptor is equal to IR_a, receptors in the IR state may become active upon Ca^{2+} binding to its activating site. The sum $IR + IR_a$ thus represents the fraction of "activatable" receptors and is referred to as IR_{able}. At any time, this fraction is given by

$$IR_{able} = (1 - R_{des})\left(\frac{I}{K + I}\right). \quad [3]$$

The fraction of active receptors is given by

$$IR_a = \frac{IR_{able}}{1 + \frac{K_{act}^{n_a}}{C_{dom}^{n_a}}}. \quad [4]$$

Starting from a previously established stationary state, we assume that, when a perturbation is applied to the system—e.g., when the concentration of InsP_3 or Ca^{2+} is suddenly changed—InsP_3 binding and Ca^{2+} binding to the activating site instantaneously reach equilibrium. At this very early moment, IR_{able} is computed, via Eq. 3, from the fraction of desensitized receptor resulting from the Ca^{2+} concentration in the domain before perturbation (Eq. 2). The transient initial state, referred to as the "peak" situation, is of special interest because it describes the immediate response of the system to a certain stimulation before the inhibiting reaction develops. This peak situation is characterized by a certain new fraction of IR_a (open state) that depends on the new Ca^{2+} concentration in the intermediate domain. Conversely, however, the channel activity governs the net Ca^{2+} flux and, thus, the Ca^{2+} concentration in this domain: the net Ca^{2+} flux through the InsP_3-dependent barrier between lumen and intermediate domain must be equal to the net Ca^{2+} flux through the fictitious barrier between intermediate domain and cytosol:

$$k_1(b + IR_a)(C_{lumen} - C_{dom}) = k_2(C_{dom} - C_{cyto}). \quad [5]$$

In this equation, k_1 and k_2 are equivalent to permeability coefficients, and $k_1 b$ accounts for a basal Ca^{2+} flux obtained in the absence of InsP_3. If ρ is defined as the ratio k_2/k_1, Eq. 5 may be rewritten as

$$IR_a = \rho \frac{C_{dom} - C_{cyto}}{C_{lumen} - C_{dom}} - b. \quad [6]$$

Given a certain fraction of activatable receptor IR_{able}, IR_a and C_{dom} must obey Eqs. 6 and 4. Eliminating IR_a by combining Eq. 6 with Eq. 4, a simple but important relationship between the fraction of activatable receptors and the Ca^{2+} concentrations in the different compartments is obtained:

$$IR_{able} = \left(\rho \frac{C_{dom} - C_{cyto}}{C_{lumen} - C_{dom}} - b\right)\left(1 + \frac{K_{act}^{n_a}}{C_{dom}^{n_a}}\right). \quad [7]$$

It has to be noticed that this formalism describes the kinetic behavior of a single Ca^{2+} pool containing a homogeneous population of $InsP_3$ receptors.

Relationship Between Ca^{2+} Flux and Activatable Receptor Fraction: Threshold for $InsP_3$ Activation. Eq. 7 allows the computation of IR_{able} as a function of C_{dom}. In Fig. 2, the results of such computation are plotted as C_{dom} (right-hand scale) versus IR_{able}. As the net Ca^{2+} flux is proportional to $C_{dom} - C_{cyto}$ (Eq. 5), the plot in Fig. 2 also permits the visualization of the dependence of the Ca^{2+} flux (left-hand scale) on the fraction of receptors in the activatable form. If Ca^{2+} binds to the activating site with positive cooperativity ($n_a > 1$) and if adequate parameter values are used, the response curve exhibits an S shape. The S shape of the curve implies the existence of threshold values for IR_{able}. For an initial state with very low IR_{able} value, the Ca^{2+} flux is low (lower branch of the S curve). If IR_{able} is increased, for instance by increasing the concentration of $InsP_3$, the flux is not significantly modified up to the moment when IR_{able} exceeds the upper threshold value. At this point, the Ca^{2+} flux abruptly switches to a high activity level (upper branch of the S curve). Because of the simple relationship between IR_{able} and $InsP_3$ (Eq. 3), the dependence on $InsP_3$ of the peak Ca^{2+} flux presents an S shape resembling the shape of the curve shown in Fig. 2. Therefore, a significant stimulation of the Ca^{2+} flux requires $InsP_3$ concentrations higher than a threshold value. This is a true threshold, reminiscent of that described in oocytes (17).

Biphasic Kinetics of $InsP_3$-Induced Ca^{2+} Release and Quantal Release. During $InsP_3$-stimulated Ca^{2+} flux, the high concentration of Ca^{2+} in the intermediate domain slowly induces channel desensitization. The progressive Ca^{2+}-induced formation of desensitized receptors leads to a slow decrease of IR_{able} (Eq. 3) and channel activity. Then, this activity abruptly returns to a quasi basal value when IR_{able} falls below a certain "lower threshold" value. Note that this abrupt reduction in Ca^{2+} flux corresponds to an abrupt reduction in C_{dom}, which falls below the dissociation constant for activation (right-hand scale in Fig. 2).

The simulation in Fig. 3*a* shows that, as can be expected from the above analysis, addition of $InsP_3$ induces a first short phase of fast Ca^{2+} release followed by a prolonged phase of slow Ca^{2+} efflux. The rate of this final slow efflux only sluggishly depends on $InsP_3$. Thus, a submaximal concentration of $InsP_3$ rapidly mobilizes only a fraction of the $InsP_3$-sensitive Ca^{2+} pool, the extent of which depends on the $InsP_3$ concentration. This behavior is reminiscent of the so-called quantal release (see refs. 1, 3, and 4 for review), but

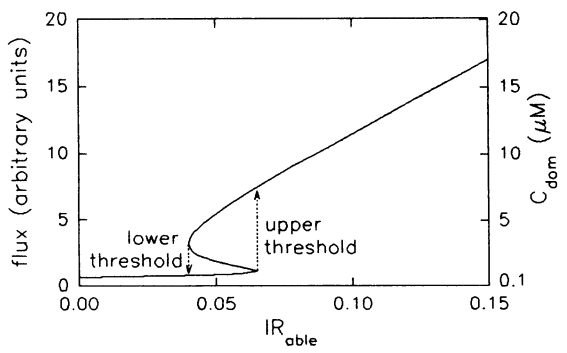

FIG. 2. Control of Ca^{2+} flux by activatable receptor fraction. The numerical values of the parameters used for this simulation are as follows: $C_{cyto} = 0.1$ μM, $C_{lumen} = 1$ mM, $K_{act} = 3$ μM, $n_a = 3$, $K_{inh} = 1$ μM, $n_i = 4$, $\rho = 9$, and $b = 0.006$. Ca^{2+} flux (left-hand scale) and C_{dom} (right-hand scale) are plotted as function of IR_{able}. The S-shaped response curve is characterized by two thresholds at which transitions between lower and upper branches of the curve occur (dotted arrows).

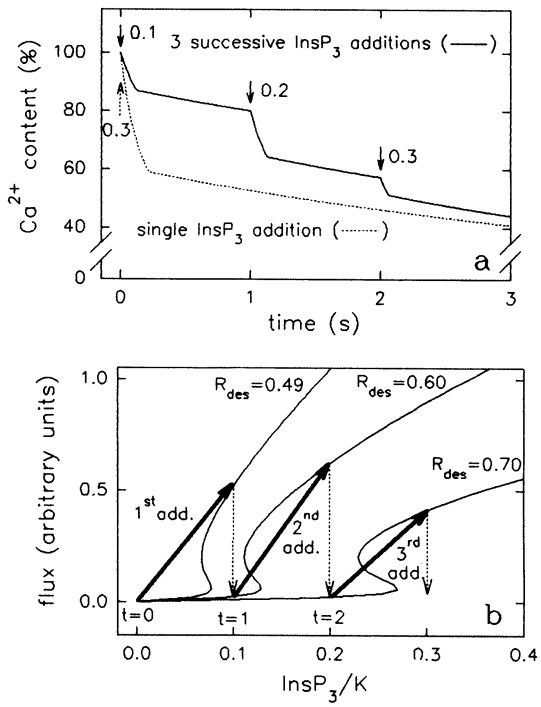

FIG. 3. Incremental detection of successive $InsP_3$ additions. The numerical parameter values for this particular simulation are as follows: $C_{cyto} = 0.1$ μM, initial $C_{lumen} = 1$ mM, $K_{act} = 0.3$ μM, $n_a = 3$, $K_{inh} = 0.1$ μM, $n_i = 4$, $k_- = 0.02$ s^{-1}, $\rho = 80$, $k_2 = 3000$ s^{-1}, and $b = 0$. (*a*) Time course of the residual Ca^{2+} content of $InsP_3$-sensitive pool, either after three successive additions of $InsP_3$ at $t = 0$, 1, and 2 s, respectively ($InsP_3/K = 0.1$ each time; solid arrows and line) or after a single addition of $InsP_3$ at $t = 0$ ($InsP_3/K = 0.3$; dotted arrow and line). The cytosolic Ca^{2+} concentration is assumed constant. (*b*) Dependence of the Ca^{2+} flux on $InsP_3$ concentration at $t = 0$, 1, or 2 s in *a*. The shape and position of the S curve are governed by the current value of the desensitized channel fraction R_{des} at the moment of $InsP_3$ addition. Boldface arrows indicate $InsP_3$-induced transitions from a low to a high Ca^{2+} flux. Dotted arrows depict the time-dependent flux decrease due to limited channel desensitization.

in our model, Ca^{2+} leaks out of a single pool with a well-defined homogeneous population of $InsP_3$ receptor.

Limited Channel Desensitization and Incremental $InsP_3$ Detection. The main reason why channel desensitization was generally not believed to account for the biphasic kinetics of $InsP_3$-induced Ca^{2+} release was that a subsequent addition of $InsP_3$ was able to provoke the mobilization of a larger amount of Ca^{2+} (3). However, the model proposed in this work deals with a kind of desensitization that does not involve *complete* inhibition of the channels. For instance, after addition of 0.1 $InsP_3$ (in K units) in Fig. 3*a*, 40% of the receptors are still activatable after the Ca^{2+} flux has stopped (Fig. 3*b*). Thus, as shown in Fig. 3*a* and interpreted in Fig. 3*b*, renewed Ca^{2+} flux is produced when more $InsP_3$ mobilizes from this reserve an IR_{able} fraction higher than the new upper threshold. These time courses are compatible with the incremental detection phenomenon previously described (3). Contrary to the general tenet (5), this study therefore proposes that quantal Ca^{2+} release and incremental $InsP_3$ detection may be the consequence of limited channel desensitization.

Indirect Effect of Luminal Ca^{2+} Concentration. The most remarkable and unexpected property of this model showed up when we studied how luminal Ca^{2+} alters the Ca^{2+} flux (Eq. 7). As shown in Fig. 4, a change of C_{lumen} modifies the position of both the lower and the upper threshold values. Starting from, for example, 1 mM luminal Ca^{2+}, if C_{lumen} is decreased, the threshold values are shifted to the right, and

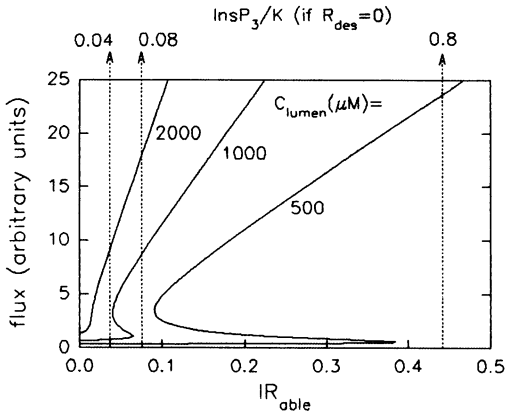

FIG. 4. Effect of luminal Ca^{2+} concentration on the dependence of Ca^{2+} flux on IR_{able}. The parameter values are the same as in Fig. 2, except C_{lumen}, which varies as indicated. Dotted arrows indicate three test values for IR_{able}, with the corresponding values for $InsP_3/K$, assuming that channels are initially not desensitized (R_{des} = 0).

the curve exhibits a more pronounced S contour. The upper branch of the curve is also lower because of the smaller Ca^{2+} gradient. Three particular concentrations of $InsP_3$ have been chosen (dotted vertical lines) to analyze the dependence of $InsP_3$-induced peak flux on the pool content: $InsP_3$ is unable to trigger significant efflux as long as the luminal Ca^{2+} does not exceed a critical value, which depends on the $InsP_3$ concentration chosen.

Biphasic kinetics of Ca^{2+} release and incremental detection have been presented above as being the consequence of channel desensitization in a homogeneous population of pools. A paradoxical property of the model is that these biphasic kinetics can now also be explained as resulting from the decrease in luminal Ca^{2+} concentration, the concomitant right shift of the lower threshold for IR_{able} and $InsP_3$, and the abrupt transition from a high to a low Ca^{2+} efflux rate. Thus, even in the absence of desensitization, the model can generate biphasic kinetics of Ca^{2+} release because of the reduction in luminal Ca^{2+}. This is reminiscent of the steady-state model proposed by Irvine (6). However, it must be emphasized that, in our case, the effect of luminal Ca^{2+} is indirect in the sense that it is mediated by the Ca^{2+} ions flowing through the channel, not by ions binding on the luminal side of the channel.

Heterogeneity of the $InsP_3$-Sensitive Pools. The major implication of the above described dependence on luminal Ca^{2+} of the $InsP_3$ sensitivity of Ca^{2+} release is that, as the Ca^{2+} contents of the different $InsP_3$-sensitive pools most probably vary due to different pump-to-leak ratios for each pool, a heterogeneous population of pools with different sensitivities for $InsP_3$ is generated by our model. Thus, our model may also explain quantal release and incremental detection in a third way—i.e., on the basis of Ca^{2+} pool heterogeneity, but without invoking the segregation of different $InsP_3$ receptors in different pools.

It was shown that stimulation of Ca^{2+} efflux requires that the $InsP_3$ concentration exceeds a certain threshold value. However, only a few experimental studies have documented such a steep curve, and the corresponding degree of cooperativity in the $InsP_3$-induced response is rather variable (for review, see ref. 4). This fact might be explained by considering that the heterogeneity of pools with respect to their Ca^{2+} content implies a dispersed distribution of thresholds for $InsP_3$. Heterogeneity might mask many different threshold values, and the apparent cooperativity of overall sensitivity to $InsP_3$ might be meaningless.

Effect of Cytosolic Ca^{2+} Concentration, and Interplay Between Luminal and Cytosolic Ca^{2+}. Another property of the model is that, like luminal Ca^{2+}, free cytosolic Ca^{2+} may indirectly control the Ca^{2+} flux by shifting the threshold values. For instance, lowering the cytosolic Ca^{2+} concentration from 0.1 μM to 0 moderately displaces the upper threshold value for IR_{able} to an upper limit (Fig. 5*a*). Thus, if the $InsP_3$ concentration used is sufficient to produce an IR_{able} fraction higher than this upper limit, the peak Ca^{2+} flux is apparently not controlled by the free cytosolic Ca^{2+}. This is a paradox, as activation of the channel by Ca^{2+} is part of the model. However, the sensitivity to cytosolic Ca^{2+} is recovered at lower $InsP_3$ concentrations (Fig. 5*b*). This sensitivity is also recovered at lower luminal Ca^{2+} concentrations (Fig. 5*b*). This remarkable behavior of our model might be the basis for the puzzling experimental observation that $InsP_3$-induced efflux exhibits a higher sensitivity to the stimulatory effect of cytosolic Ca^{2+} when the Ca^{2+} content of the pools is lower (18).

DISCUSSION

Analysis of the model described above reveals the possible existence of multiple steady states for stimulated Ca^{2+} flux. S-shaped response curves classically lead to development of all-or-none responses, the intermediate branch of the S describing unstable steady states (19). In our case, the transition from a state of low activity to a state of high activity for the $InsP_3$ receptor occurs when binding of $InsP_3$ produces

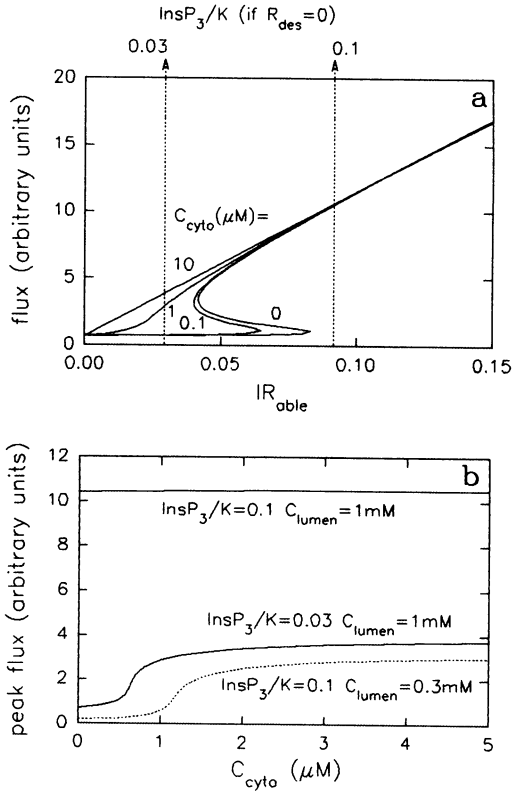

FIG. 5. Effect of cytosolic Ca^{2+} concentration. The parameter values are the same as in Fig. 2, except C_{cyto}, which varies as indicated. (*a*) Dependence of Ca^{2+} flux on IR_{able} for different cytosolic Ca^{2+} concentrations. Dotted arrows indicate two test values for IR_{able}, with the corresponding values for $InsP_3/K$, assuming that channels are initially not desensitized (R_{des} = 0). (*b*) Dependence of the peak flux on cytosolic Ca^{2+} concentration observed with these two different $InsP_3$ concentrations (solid lines). The dependence is also shown for $InsP_3/K$ = 0.1 when the Ca^{2+} content of the pool is reduced (C_{lumen} = 0.3 mM; dotted line).

a fraction of activatable receptor exceeding a critical value (upper threshold). The reciprocal transition occurs when the fraction of activatable receptors becomes less than another critical value (lower threshold), for instance, because of Ca^{2+}-induced desensitization or because of a reduction in luminal Ca^{2+}. The existence of a threshold for $InsP_3$, which is reminiscent of experimental observations on Ca^{2+} signals evoked by photoreleased $InsP_3$ (17), implies that noncooperative $InsP_3$ binding to the receptor may coexist with a steep dose dependence for $InsP_3$-induced Ca^{2+} release. A second remarkable feature of the model is the possible control of $InsP_3$ sensitivity by the luminal Ca^{2+} concentration, even in the absence of any specific Ca^{2+}-binding site facing the lumen. Such control directly leads to functional heterogeneity in the population of Ca^{2+} pools because Ca^{2+} loads probably vary from one pool to another. Heterogeneity within a single cell might contribute to the spatial cytosolic distribution of the Ca^{2+} mobilization response to the $InsP_3$ stimulus, but the abrupt activation of the Ca^{2+} flux in response to a small $InsP_3$ concentration increase could be masked in heterogeneous population because of the different $InsP_3$ threshold values. A third unexpected characteristic of the model was the fact that under certain conditions, Ca^{2+}-induced activation of the $InsP_3$-sensitive channel, although intrinsic to the model, appeared not to be observable experimentally. This result of our simulations contradicts several experimental observations that concluded to a coagonist role of cytosolic Ca^{2+} in the $InsP_3$-induced Ca^{2+} release (13–15) but agrees with other reports showing no activation by Ca^{2+} (20) or the quasi-annihilation of the cytosolic Ca^{2+} stimulatory role by high Ca^{2+} loads (18). Our model might allow reconciliation of these various observations by showing that sensitivity to cytosolic Ca^{2+} depends a lot on experimental conditions. The same is true for the sensitivity to luminal Ca^{2+}, which has also been a controversial issue among experimentalists (3, 7–10). Some of the reported kinetic behaviors, like quantal release or incremental detection, may be lost if the value of one or another parameter is only slightly changed. Therefore, the observations of qualitatively distinct kinetic behaviors obtained under different experimental conditions are not inconceivable.

The proposed model has been elaborated on the basis of a few simple concepts—namely, the existence of activating and inhibiting Ca^{2+}-binding sites situated in an intermediate domain. More complex models could be developed (for instance, by introducing various dissociation constants for $InsP_3$ binding to the different forms of receptor or by allowing simultaneous Ca^{2+} association to inhibitory and activating sites). Nevertheless, the current mathematical formulation of the model has the merit of proposing a conceptual basis to explain several intricate observations and reconcile divergent experimental results. What remains to be established is the exact nature of the postulated intermediate domain. It might correspond to the aqueous inner compartment physically defined by the cytosolic part of the channel protein or, alternatively, to a cytosolic submembrane small volume around the channel mouth in which the local Ca^{2+} concentration can be very different from the bulk Ca^{2+} (21–24). Both interpretations are compatible with the proposed model.

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